Current expectations for laboratory testing and adverse smallpox vaccine reactions

- Poxvirus Section (DVRD/NCID/CDC)

- World Health Collaborating Center for Smallpox and other Poxvirus Infections

Inger Damon: Chief

Russell Regnery

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Poxvirus intro:

2 Subfamilies:

- **Chordopoxvirinae (vertebrate poxviruses)**
  - **Orthopoxvirus (variola, vaccinia, cowpox, monkeypox, raccoonpox, camelpox, skunkpox, volepox, ectromelia, taterapox)**
  - **Parapoxvirus (orf, pseudocowpox, …)**
  - **Avipoxvirus (canarypox, fowlpox…)**
  - **Capripoxvirus (goatpox, lumpy skin disease…)**
  - **Leporipoxvirus (myxoma, fibroma…)**
  - **Molluscipoxvirus (molluscum contagiosum)**
  - **Yatapoxvirus (tanapox, Yaba monkey tumor)**
- **Entomopoxviridae (insect poxviruses)**

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Orthopoxviruses (including vaccinia and variola)

- 180 to 200 kbp double stranded DNA genomes with over 180 predicted ORFs
  - Encodes transcription and replication enzymes
  - Encodes multiple proteins aimed at evasion of immune defense molecules:
    - Soluble cytokine and chemokine binding proteins
    - Caspase inhibitors, inhibitors of protein kinase PKR
  - Cytoplasmic replication
  - Infectious forms: IMV, CEV, EEV
  - No known unique viral receptor protein
  - Host ranges vary
    - Variola (host specific…man) vs vaccinia (wide host range)
  - 350 X 270 nm brick shaped particles by cryoelectron microscopy
  - Antigenically similar; serologic cross reactivity

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Orthopoxviruses: spectrum of human disease in normal host

- **Vaccinia**, cowpox: localized infection
- **Variola**, monkeypox : systemic illness

- Camelpox: no known infections of humans
- Ectromelia: no infection of humans
- Raccoonpox, volepox, skunkpox: rare if any infection of humans with these animal pathogens

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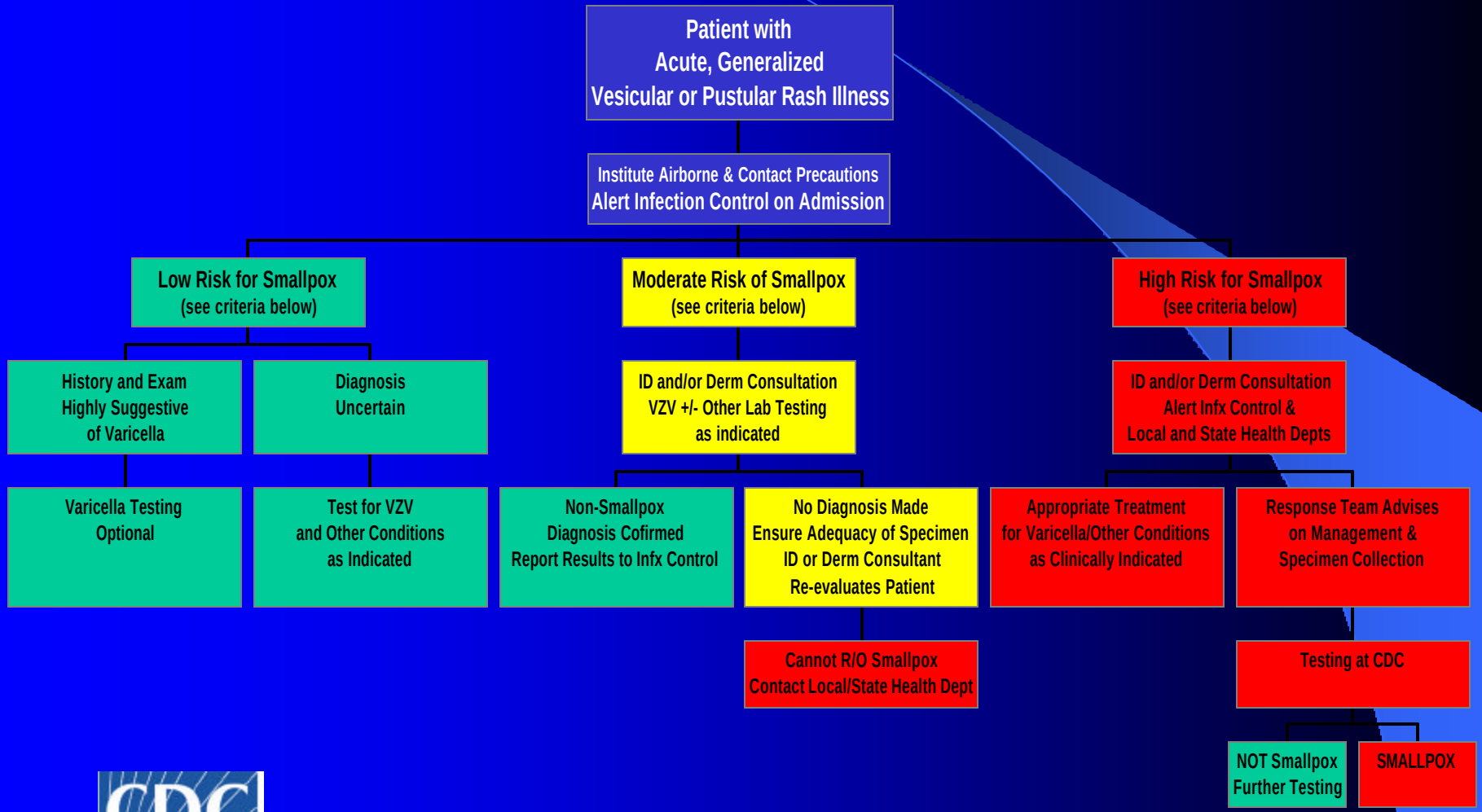
Febrile, vesicular rash illness algorithm for evaluating patients for smallpox



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Key concepts of algorithm for evaluating pts for smallpox

- Product of practical experience and partnerships
- Uses existing resources for dx and exclusion of smallpox look-a-likes (especially 1.5m cases of chickenpox)
- Provide significant diagnostic benefits, even in absence of smallpox…encourages careful dx of other rash illnesses
- Minimize number of cases that require intensive investigation: focus attention where it is justified
- Provide rapid, thorough response to highly suspect cases
- Respond with vaccination if/when diagnosis confirmed

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Smallpox dx: the Bottom Line

- To rapidly recognize and respond to the first case of smallpox quickly *without*
  - Generating high numbers of false alarms
  - Disrupting the health care and public health systems
  - Increasing public anxiety

- **Secondarily,** benefit health care (in absence of smallpox) by enhancing rash illness dx capability

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Smallpox dx rational (pre-event)

- ***Multiple samples*** **(& multiple tests) to improve predictive value for positive variola lab dx in absence of disease in nature.**

- **Multiple samples (e.g., biopsy) may provide means for dx of smallpox look-a-likes; improves Public Health understanding of what can be clinically confused with smallpox**

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Vaccinia identification: lab expectations/considerations (I)

- Most AE cases linked directly to vaccination history or contact of vaccinee.
- Vaccinia therapeutic options limited to VIG or Cidofovir (?)
- Decision to use VIG or Cidofovir made at clinical level

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Vaccinia identification: lab expectations/considerations (II)

- Identification of vaccinia in AE's will help improve Public Health understanding of AE's and vaccination risks; hopefully lead to better vaccines / vaccination strategies. Not a STAT function.
- LRN labs have means to detect vaccinia
  - But currently R-T PCR test considered by the FDA an "investigational device" or a "presumptive screening assay".
  - Test results for pt management must be confirmed at CDC.
- Rule out other possible etiologies

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Smallpox vs vaccinia: Lab tests may be similar but expectations for results and responses different

- Pre-event *smallpox* dx implies international crisis & mass vaccination
- *Smallpox* dx implies pt isolation and vaccination of contacts

- *Vaccinia AE's* expected to occur in small numbers (not the prelude to a pandemic!)
- *AE* patient care decisions based primarily on pt history and clinical considerations

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Smallpox & generalized vaccinia: Differential Diagnosis & other rash illnesses

- Varicella**
- Disseminated herpes zoster*
- Impetigo
- Drug eruptions
- Contact dermatitis
- Erythema multiforme
- Enteroviral infections
- Disseminated herpes simplex
- Scabies, insect bites
- Molluscum contagiosum (immunocompromised)

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Laboratory Testing to Rule Out Other Rash Causing Diseases

- VZV: DFA, PCR, EM, Immunohistochemistry
- Streptococcus, staphylococcus: Gram stain, rapid tests
- Drug eruptions, allergic dermatitis: skin biopsy, pathology
- Enterovirus infections: PCR, immunohistochemistry

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Laboratory Testing to Rule Out Other Rash Causing Diseases - Continued

- Herpes simplex: PCR, EM, Immunohistochemistry and Culture (with caution)
- Scabies: Evidence of organisms
- Others as indicated from clinical impression:
  - *Rickettsia*: PCR
  - Syphilis

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Varicella Rule-Out

- History and Clinical Findings
- Laboratory:
  - VZV DFA (two commercial products: Meridian Diagnostics and Chemicon)
  - PCR ("Homebrew") plus LRN supported test
  - Electron microscopy
  - Refer All specimens with questionable results to DPH Laboratory for Confirmation

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Generalized vaccinia, varicella, and smallpox: typical clinical features

	Generalized Vaccinia	Varicella (chickenpox)	Smallpox
Prodrome	Rare ??	Rare	Always
Distribution	Vac site + disseminated	Centripetal	Centrifugal
Lesions	Vac site + deep-seated pustules	Superficial vesicles	Deep-seated pustules
Progression	??	Rapid: some crusts <24 h	Slow: each stage 1-2 d
Stages of Dev't	Same (following vac) ?	Different	Same
Palms/soles	??	Extremely rare	Typical
Vac status	Recent smallpox vac or contact	Rare with VZV vac	Very rare with smallpox vac
Toxic	No	No	Yes

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Smallpox (plus vaccinia?) Lab Algorithm

Low & Moderate Risk Specimens

(Green & Yellow Box)

Level A (hospitals)* and/or

LRN B/C Laboratories

VZV, HSV DFA

VZV, HSV, Enterovirus PCR (where available)

Other appropriate diagnostic assays, including viral culture

Positive = Diagnosis of non-variola rash illness

Negative:

- Re-evaluate patient condition and assess need for dermatologic and histologic testing, including tests for erythema multiforme.
- Obtain detailed information about possible exposure to smallpox vaccines.

Tests for vaccinia and generic orthopox may be indicated. IF vaccinia -, generic orthopox +, contact CDC immediately.

- If patient symptoms progress to more closely resemble smallpox, all specimens should be referred to CDC (and regional labs when needed).

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Orthopoxvirus-specific assays for identification of:

- Vaccinia (e.g., generalized vaccinia)
- Variola (smallpox)

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Lab methods for confirmation of orthopoxvirus dx

- PCR related methods for DNA identification, e.g., real-time PCR
- Electron microscopy
- Histopathology
- Culture
- Serology
  - Antigen detection (IFA/DFA, EIA ag capture)…not yet available/licensed

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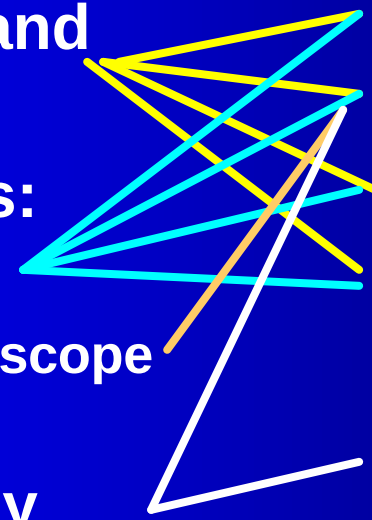
Vaccinia-related disease: *potential* samples and tests

- **Samples:**
  - Lesion ‘roofs’ and crusts
  - Vesicular fluids:
    - touch prep
    - Electron microscope grid
  - Biopsy, autopsy



- **Tests:**
  - R-T PCR (LRN)
  - Electron microscopy
  - Isolation in cell culture
  - Antigen Capture & DFA (in the future)
  - Histopath

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Sample requirements for Poxvirus *DNA* identification

- ✓Lesion ‘roofs’ and crusts
- ✓Vesicular fluids (touch prep)

- ✓Biopsy, PM autopsy
- ✓Others (e.g. CSF?)

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Sample requirements for *EM* poxvirus identification**

- ✓Lesion ‘roofs’ and crusts
  (grids subsequently made at lab)
- ✓Vesicular fluids:
  - touch prep slide (grids made at lab)
  - electron microscope grids made on- site

** requires access to EM

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Sample requirements for vaccinia (not variola*) *isolation*

- ✓Lesion ‘roofs’ and crusts
- ✓Vesicular fluids:
  - touch prep slide (reconstituted at lab)
- ✓Frozen biopsy including PM tissue (?)

- ✓**BSL-II conditions; vaccinated lab workers preferable**

***Variola referred to CDC (pre-event)**

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Sample requirements for *histopathology*

- ✓ **Biopsy or autopsy**
  formalin fixed (not frozen)

  (remember to save fresh frozen bisected or duplicate sample for isolation)

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Specimen collection

- **Vaccinia and variola specimen collection essentially the same.**

- ***Check CDC website for recent updates in orthopox specimen collections specifics:***
  - **http://www.bt.cdc.gov/agent/smallpox/response-plan/files/guide-d.pdf**
  - **Expect vaccinia specific updates in near future (first week December 2002)**

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What to collect? (I)

Vesicles:

- Use scalpel or 26 gauge needle to unroof vesicle – skin of roof goes to collection tube
  - Scrape base of vesicle with blunt edge scalpel, or wooden applicator and apply to microscope slide
- Lightly apply EM grid, shiny side down, against lesion. Repeat (X2) using more or less pressure.
- Repetitively touch a microscope slide to the lesion (touch prep)
- Allow slide, and grids to air dry for 10 minutes. Store in slide holder, and grid box, respectively

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What to collect? (II)

Biopsy: 3.5-4 mm punch biopsy, bisect lesion, or obtain 2 biopsies.

1 sample in specimen collector tube (keep cold/frozen)

1 sample in formalin (not frozen).

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Real-Time PCR assay: E9L-Vaccinia detection (Non-variola Eurasian orthopoxvirus assay)

- Samples are tested using primers and probe designed to detect Eurasian Orthopoxvirus other than variola
  - Potential human diseases detected:
    - Vaccinia **
    - Cowpox (Zoonotic disease of European origin)
    - Monkeypox (Zoonotic disease of central Africa)

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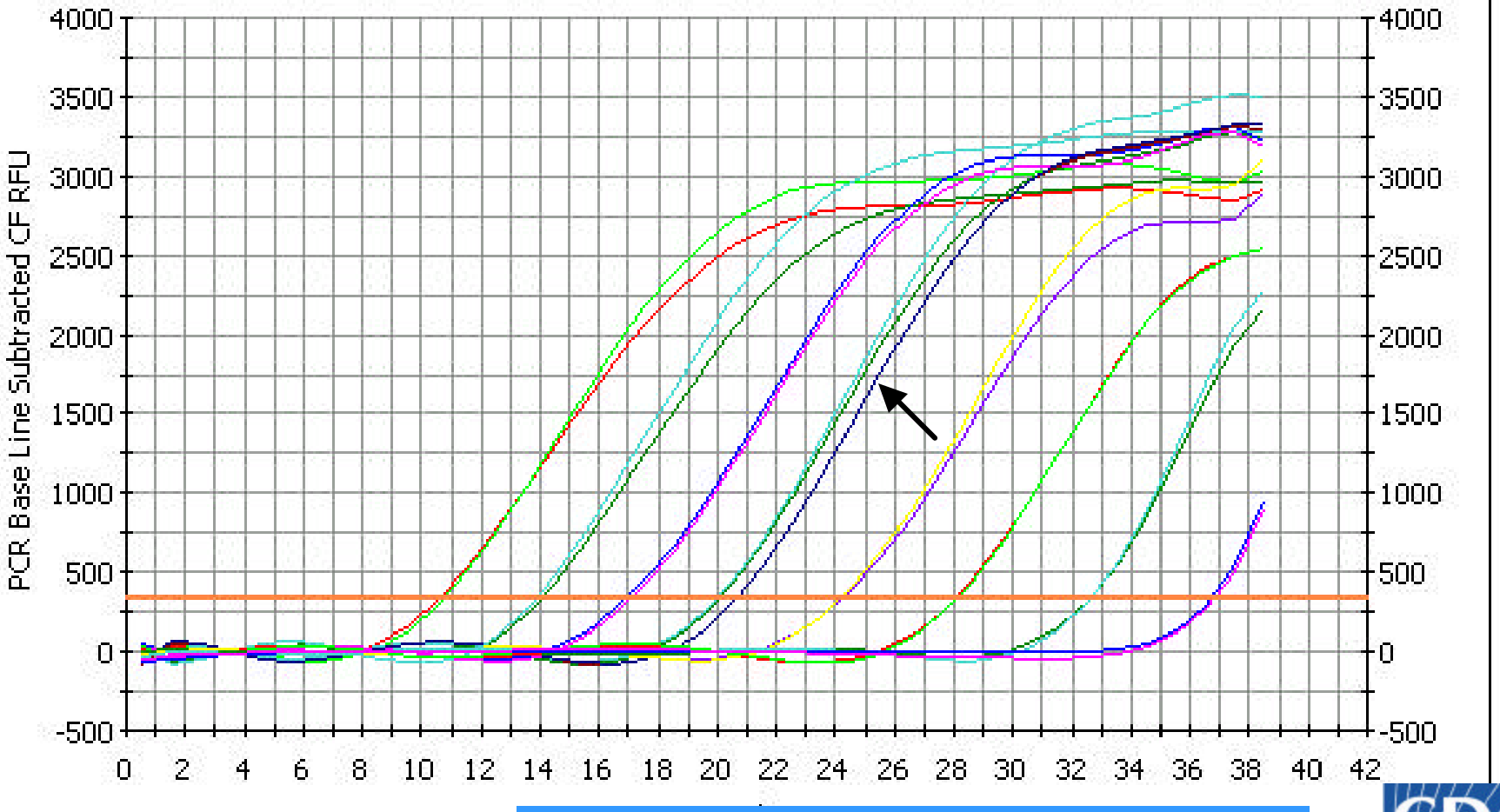
Example of calibrated, R-T PCR data



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Sensitivity of E9L vaccinia R-T PCR assay during validation at LRN labs

- 16/16 labs detected equivalent of 100 pfu vaccinia from dried, touch-prep slide…very sensitive!

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If smallpox were to re-emerge, test for vaccinia AE's would be modified to become test for variola virus DNA

- Alternate primer supplied…real-time PCR test otherwise essentially the same

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Vaccinia/orthopox and variola: real time PCR assays

- E9L: VAC, MPX, CPV(TET); variola (FAM) and ABI 7700
  - Essential gene of poxviruses
  - 16S control for inhibitors
  - Can be used to detect vaccinia (adverse event monitoring); use TET portion
  - Can be used to detect variola use FAM and TET portions (100% specificity in blind evaluation of 451 specimens)

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Orthopoxvirus generic R-T PCR test

- Different E9L gene primer/probe targets *all* human pathogenic orthopoxviruses
- Currently being validated in LRN test sites as an “environmental sample” test
- Could have potential role in identification of non-vaccinia orthopoxvirus infections
- Sensitivity expected to be as high as test for detection of vaccinia

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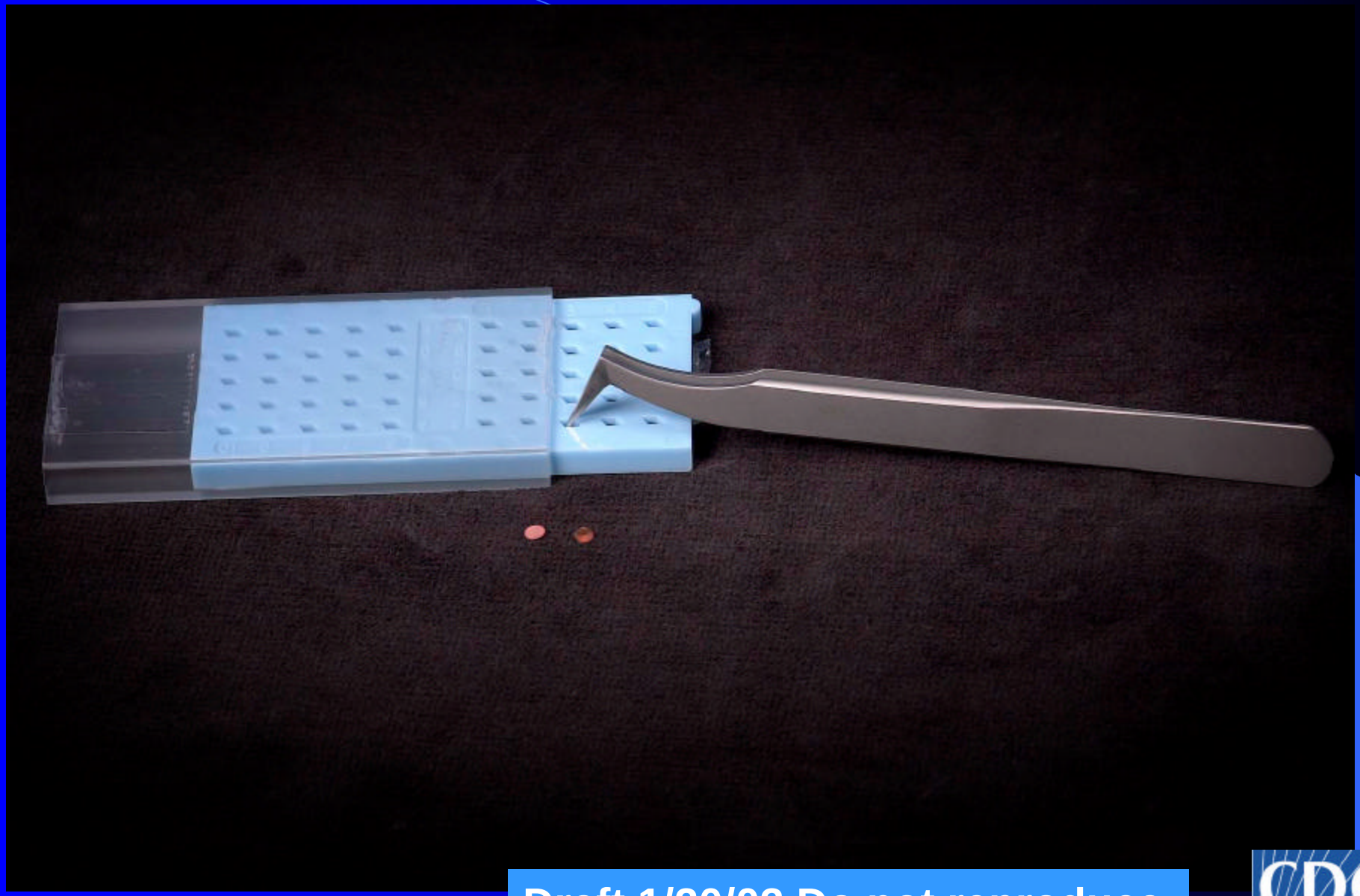

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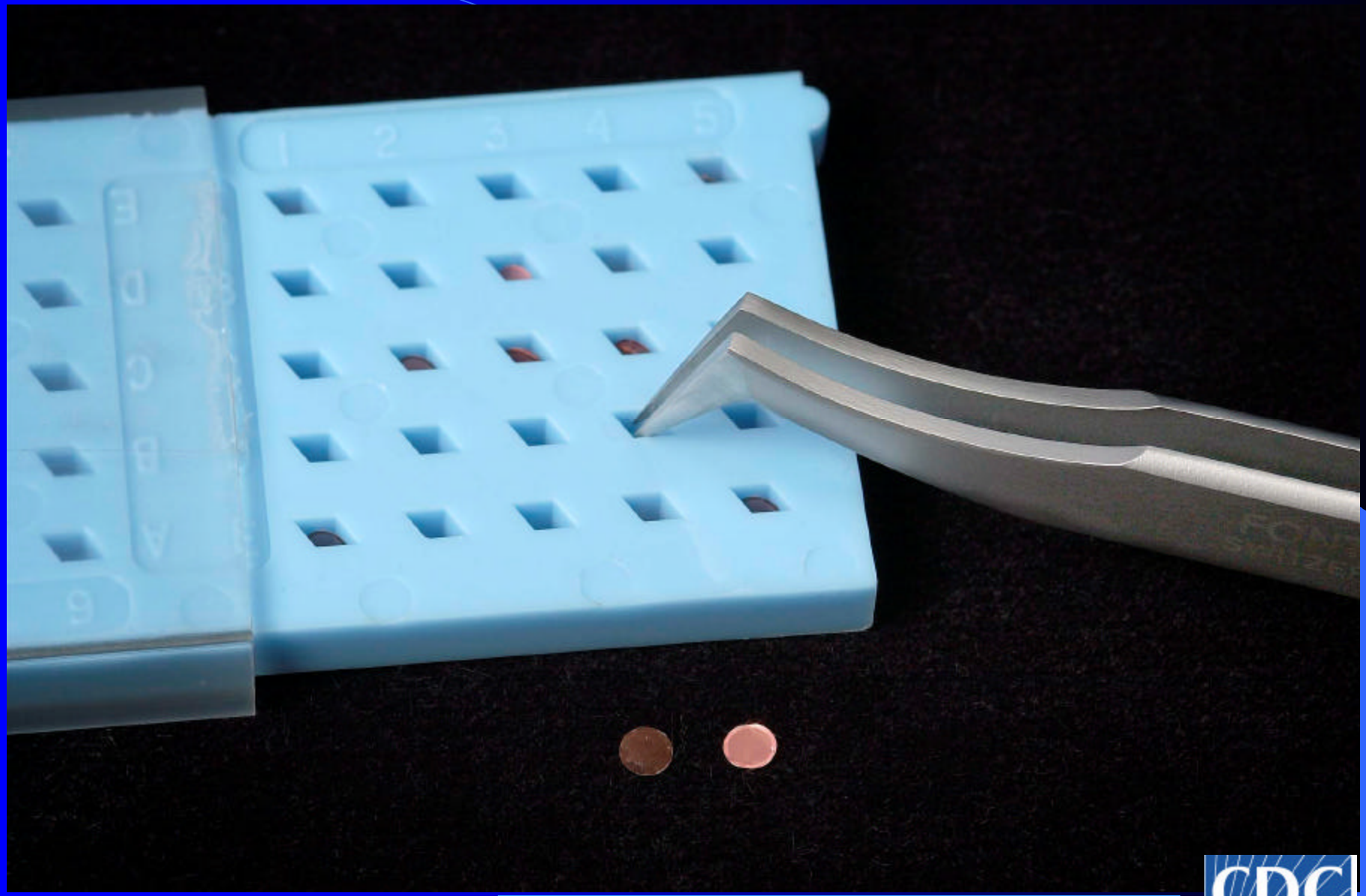

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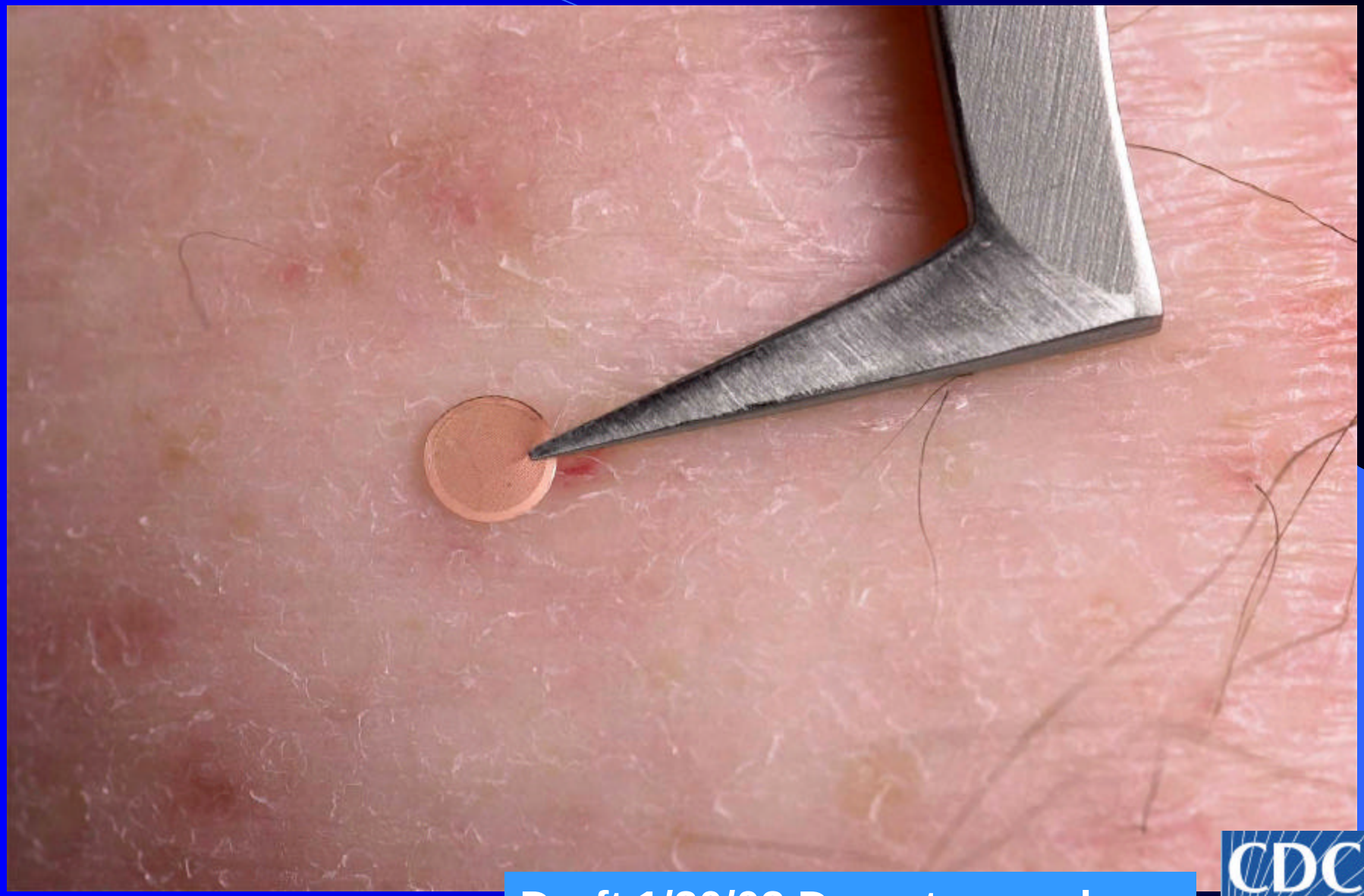

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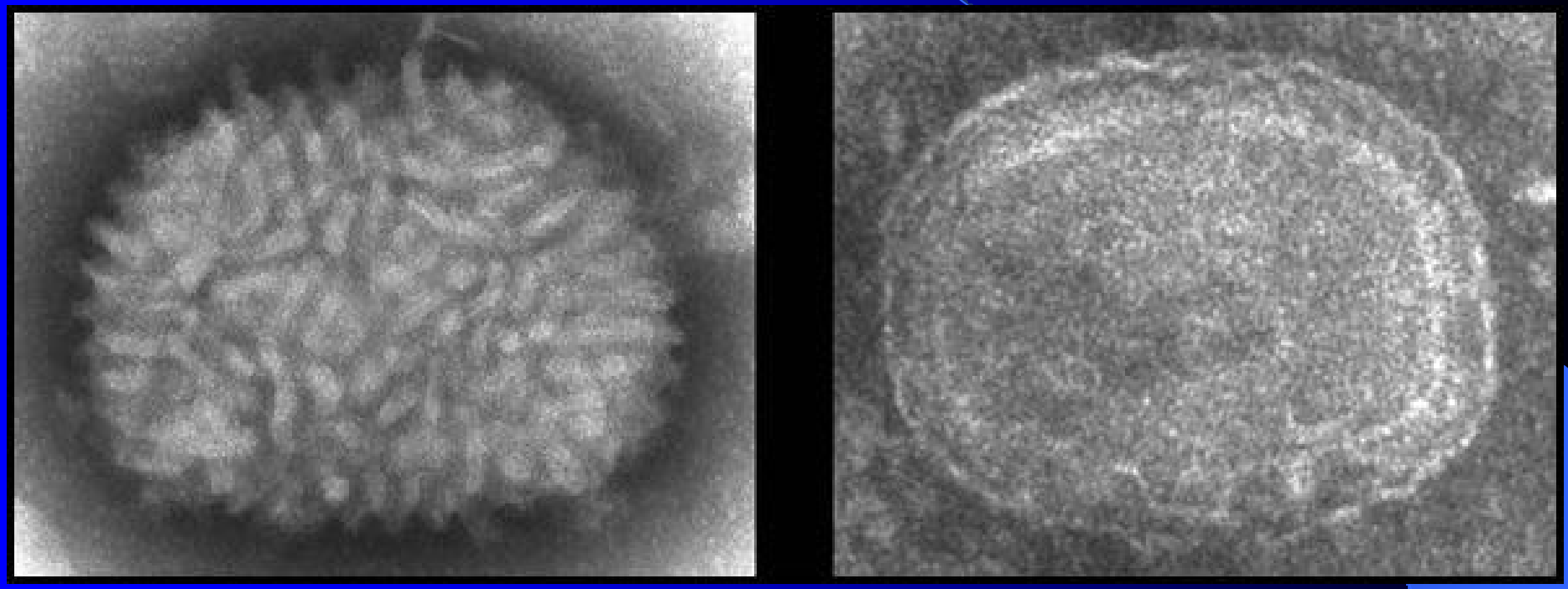
Negative Stain Electron Microscopy



vaccinia

~1/2 hour per sample (for experienced microscopist)

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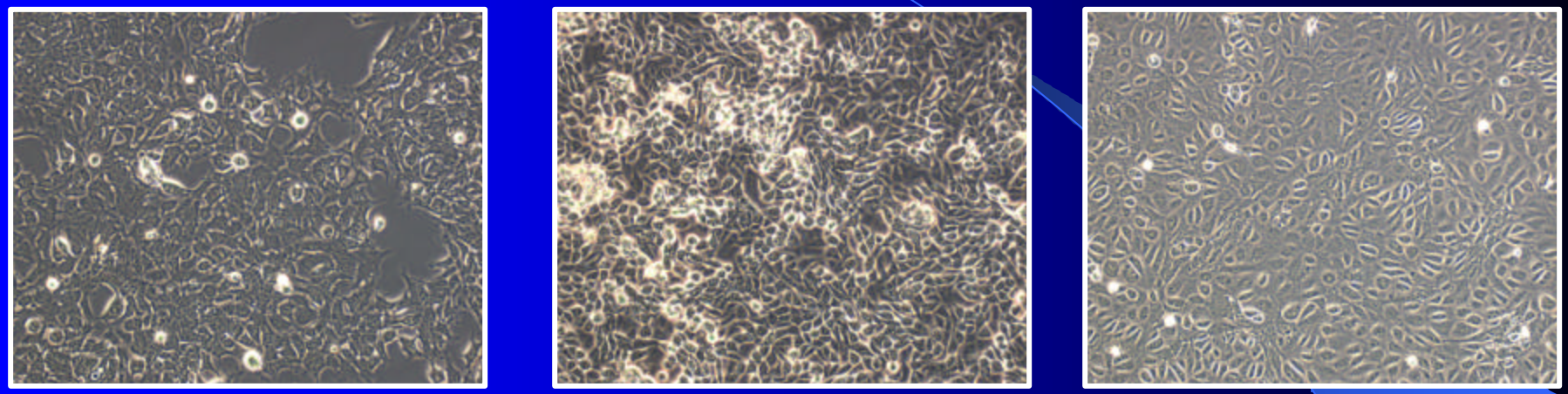
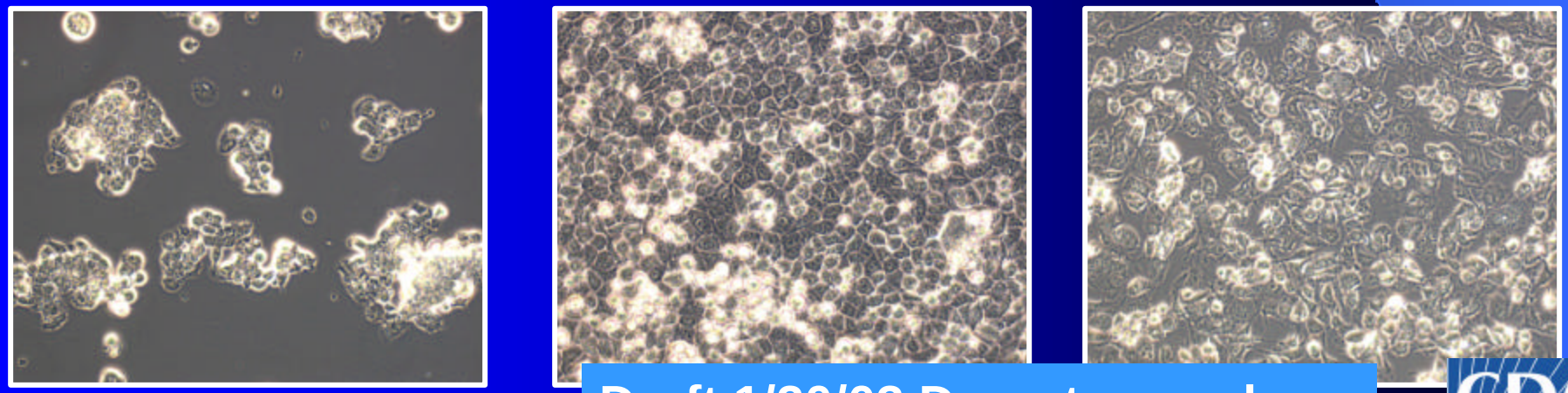
Orthopoxvirus cell culture isolation

HEK 293 | HeLa | BSC-40

Uninfected, 24 hpi



Variola (Ethiopia 17 R14-1X-72) infected, 24hpi



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Selected issues relevant to implementation of pre-event vaccinia diagnostics

- Transition research/reference laboratory methods to routine patient care
  - Distribute test protocols
  - Test validation in multiple high-tech formats
  - Evaluation/validation of tests under ‘field’ conditions
  - Vaccinate testing personnel
  - QA/QC, Proficiency testing
- Reagents production/dispersal
  - Approval to use
- Development of vaccinia test algorithms (in conjunction with clinical algorithms)
- Involving private sector in rapid test development, licensure, deployment

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Where to send orthopox specimens?

- Suspect **vaccinia adverse events** specimens that require identification of vaccinia go to closest **Laboratory Response Network** (LRN) laboratory.
  - Contact your State Public Health Lab Director for shipping address
  - All state and regional LRN labs can do real-time PCR for identification of vaccinia in AE's

- Specimens from persons with high suspicion of smallpox dx: Refer to *Rash, Vesicular Disease Algorithm*. Specimens go both to selected LRN with smallpox surge potential (contact CDC) and CDC simultaneously.

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Specimen transport

- Standard dx specimen shipping guidelines available (subject to change): http://www.bt.cdc.gov/labissues/PackagingInfo.pdf
- Serum, if collected, should be refrigerated and shipped
  - If spun and separated on site, freeze
- Formalin fixed material should be shipped at room temperature DO NOT FREEZE
- EM grids should be shipped at room temperature
- All other virus containing material should be stored and shipped frozen, unless it will be overnight shipped, then room temperature or refrigerated
- Keep all virus containing material out of direct sunlight

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Past and Future

- What about the Past (when low tech worked)?
  - During smallpox epidemics *clinical* dx drove immediate medical response
  - Electron microscopy
  - Gel-diffusion serologic antigen detection

- Future? (dx development evolving rapidly)
  - Additional PCR targets (e.g., HA gene)
  - Antigen capture and DFA

- Post-event, expectations for dx would change

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For More Information

- CDC Smallpox website

  www.cdc.gov/smallpox

- National Immunization Program website

  www.cdc.gov/nip

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